Original Article

HAEMATOLOGICAL PROFILE OF ANTI-RETROVIRAL THERAPY (ART)-NAIVE HIV POSITIVE CHILDREN IN ENUGU, NIGERIA.

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ABSTRACT

BACKGROUND: Haematological complications such as anaemia, neutropenia and thrombocytopenia are associated with Human Immunoeficiency Virus (HIV) disease progression and reduced survival. These have been documented to be the second most common cause of morbidity and mortality in infected children. This study sought to assess the haematological parameters in HIV positive Anti-retroviral therapy (ART)-naïve children and compare with the HIV negative children in order to discover ways of maximizing outcomes and value from ART when eventually indicated.

METHODS: This was a prospective observational study done in 2016 at University of Nigeria Teaching Hospital, Enugu, involving a total of 200 children. Blood samples were collected for full blood count (FBC) and Cluster of Differentiation 4 (CD4) counts. Data generated were analyzed with Statistical Package for Social Sciences (SSPS) version 22.

RESULTS: The prevalence of anaemia was 60% among HIV-infected children.Hemoglobin levels unlike leucocyte and platelet counts were significantly associated with HIV-infection. CD4 count was inversely associated with anaemia. Similarly the incidence of anaemia but not neutropenia and thrombocytopenia was also inversely and significantly associated with the ages of the children.

CONCLUSION: Anaemia was prevalent among HIV infected children and is inversely associated withCD4 count. Haemoglobin concentration estimations can therefore be considered as one of the criteria for commencement of anti-retroviral medications especially in resource poor centers where CD4 count is not readily available.

KEYWORDS: HIV, Antiretroviral therapy naïve, Anaemia, Thrombocytopenia, CD4 count, Children.

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INTRODUCTION

The Human Immunodeficiency Virus (HIV) is one of the most important infections of this century. It is probably one of the diseases with multiple impacts on persons, families, communities and the entire society. Over 3 million children were noted to be living with HIV around the world at the end of 2013.[1] Haematological complications have been documented to be the second most common cause of morbidity and mortality in HIV positive persons.[2] A variety of haematological manifestations, sometimes life-threatening, is seen at every stage of HIV infection impairing the quality of life of these patients.[3]These pose a great challenge in the comprehensive management of such patients.[4-5] Haematological abnormalities have been documented as strong independent predictors of morbidity and mortality in HIV infected children.[3]Although it is neither part of the criteria for initiating therapy nor used by the World Health Organization (WHO) for staging HIV, peripheral blood cell abnormalities with an abnormal haemogram are important prognostic tools for morbidity in HIV infection and AIDS.[3] The presence of these complications in HIV infected ART-naïve patients usually result in poor treatment outcome when ART becomes indicated and strongly predict poor prognosis and increased mortality.[6]. Infection with HIV ultimately leads to profound immunosuppression in which patients may present with various AIDS defining clinical conditions (which are a set of diseases associated with AIDS) that may impact negatively on his quality of life. Impaired haematopoiesis, immune-mediated cytopenias and altered coagulation caused by increasing viral load as the disease progresses may account for the

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hematological abnormalities seen in HIV infection. These abnormalities may occur as a result of the infection, as sequelae of HIV-related opportunistic infections or malignancies.

Anaemia is the most common haematological abnormality in HIV-infected patients.[7-9] The consequences of untreated anaemia may lead to multisystem disabling symptoms and fatigue, exhaustion, increased risk of HIV dementia and poor quality of life.[10] Thrombocytopenia is also a frequent complication of HIV infection.[9,11-12]The possible mechanisms that have been reported are immune-mediated destruction of platelets by antibodies and cross-reacting antibodies that are directed towards HIV proteins.[13]

Thrombocytopenia has also been identified as a strong independent predictor for mortality in HIV positive patients.[14] Neutropenia is the most common leucopenia occurring in HIV infected individuals.[9]HIV infection suppresses the bone marrow and leads to decreased levels of granulocyte colony-stimulating factor.[9] Low CD4 count has been associated with anaemia, neutropenia and thrombocytopenia.[15] . HIV preferentially infects CD4 cells causing their destruction. This study was to assess those haematological parameters in ART-naïve HIV positive patients and compare them to HIV negative subjects.

Africa is the epicenter of the HIV pandemic as reported the World Health Organization (WHO) and Sub Saharan Africa contributes about 60% of the burden worldwide. About 3.4 million Nigerians were living with the virus in 2012 which represents a national prevalence of 5.8%.[16]
In Nigeria and most Sub-Saharan Africa, HIV patients are mostly dependent on donor medications from the developed nations hence there is need for continuous research to improve the treatment outcomes and add value on the huge resources spent on the treatment of HIV infection and AIDS disease

METHODOLOGY

Study setting and study population

This was a prospective observational study conducted at the HIV clinic of the University of Nigeria Teaching Hospital, Enugu from July 13, 2015 to November 20, 2015. HIV infected children (1 to 12 years) who were not yet on antiretroviral medications and whose parents consented, were recruited consecutively into the study. Febrile and malnourished children were excluded from the study.

Ethical considerations

Ethical clearance was obtained from the Ethical and Research committee of University of Nigeria Teaching Hospital before commencement of the study. An informed consent form was filled and signed by all consenting caregivers.

Data collection

Medical and demographic information were obtained from all the study participants using a proforma. Blood for full blood count was collected using an EDTA bottle. Samples were then analyzed using an auto analyzer. Two weeks later a second sample was collected from the same participants and those with consistent values on both occasions were enrolled. Anaemia was defined as hemoglobin level less than 10 grams/deciliter. The CD4 count was done using the Becton Dickinson FACS Calibur system.

Statistical analysis

The data were then analyzed with Statistical Package for Social Sciences (SPSS) version 22 of the IBM Corporation. Descriptive statistics were used to summarize categorical and continuous variables. Median values of absolute neutrophil count and platelet (skewed variables) were comparedbetween tests and age/sex matched controls using Mann-Whitney U test while mean values of haemoglobin concentration were compared between the same groups using Student's t-test. Relationships between age, CD4 count and haematological parameters were determined using correlation and linear regression. Associations between HIV statuses, different levels of CD4 count and haematological abnormalities were tested using logistic regression and Chi-square. The statistical significance was set at $p < 0.05$. Results were presented in tables, charts and scatter plots.

RESULTS

A total of 200 children (100 HIV Positives and 100 controls) were enrolled into the study, all matched for sex and age. There were 48 males and 52 females with ratio of approximately 1:1 (Table i). The age ranged from 1 to 12 years with a mean ± Standard Deviation (SD) of 5.59 ± 3.25 years.

Table 1: Sex and age distribution of all study participants

	Infected		Control	
Age (group)	Male	Female	Male	Female
1 – 3	19 (39.6)	14 (26.9)	19 (39.6)	14 (26.9)
4 – 6	9 (18.8)	15 (28.8)	9 (18.8)	15 (28.8)
7 – 9	14 (29.2)	16 (30.8)	14 (29.2)	16 (30.8)
10 – 12	6 (12.5)	7 (13.5)	6 (12.5)	7 (13.5)
Total	48 (100)	52 (100)	48 (100)	52 (100)

Among the study participants, the absolute neutrophil counts (ANC) ranged from 572 to 18711 cells/mm^3 with mean ± SD of 3176 ± 2469 cells/mm^3 while amongst the controls, the ANC ranged from 105 to 12450 cells/$mm^3$with mean ± SD of 3966.14 ± 2646.14 cells/$mm.^3$) The median ANC for the HIV positive subjects 2428.50) and the controls (3320.00) were not significantly different. (p = 0.115).

Mean platelet levels in infected children were 323900.00(SD ± 129147.67) cells/mm^3, range 26000 -708000 cells/mm^3 while in the controls, mean was 312000(SD ± 290000.00 cells/mm^3) and range 112000 -975000 cells/mm^3. The difference in the median platelet levels for the test subjects and the test controls were not significantly different. (p = 0.226).

The mean haemoglobin concentrations in infected children and uninfected children were9.44g/dl(SD± 1.44) range 5.20 -12.20g/dl and 10.67g/dl (SD ± 1.24), range 6.60 - 15.30g/dl respectively. There was a normally distributed haemoglobin concentration values in both groups.

Table II shows the prevalence of haematological abnormalities among the study participants. The prevalence of anaemia was 60% and 23% among the infected and uninfected respectively.(Table iii) Anaemia is 5 times more significantly associated with the HIV infection than test controls (p < 0.001, OR = 5.022, 95% C.I = 2.718 – 9.279). Neutropenia was seen in 18 sero-positive (11 males and 7 females) and in 14 sero-negative (9 males and 5 females) study participants (p = 0.441). The prevalence of thrombocytopenia is 7% among the test subjects and 6% among the test controls. (p = 0.774).

Table 2: Relationship between prevalence of haematologic abnormalities in HIV infected subjects and controls

	Infected	Control	p value	OR	95% C.I for OR
Haemoglobin					
Anaemia	60	23	< 0.001	5.022	2.718 – 9.279
Normal	40	77			
Neutrophil Count					
Neutropenia	18	14	0.441	1.348	0.630 – 2.887
Normal	82	86			
Platelet count					
Thrombocytopenia	7	6	0.774	1.179	0.382 – 3.641
Normal	93	94			

Haemoglobin, unlike neutrophil and platelet concentration had a significant positive but weak relationship with age in infected children (p < 0.001, r = 0.348)(Table iii)

Table 3: Relationship between age and haematological parameters in HIV infected participants.

	Statistics	Neutrophil (cell/mm3)	Platelets (cell/mm3)	Haemoglobin (g/dl)
Age	Pearson Correlation (r)	-0.110	-0.077	0.348
	p value	0.277	0.449	< 0.001*
	N	100	100	100

No significant relationship was found with neutrophil levels, platelet levels and haemoglobin concentration among the controls.

The overall minimum CD4 count was 4 cell/mm3 and the maximum was 2098 cells/mm3 with a median CD4 count of 350 cells/mm3. Eighteen percent (18/100) of the study population had CD4 counts of < 200 cells and 19% (19/100) of them had CD4 counts <between 200 and 500 cells. Majority of the study population 63% (63/100) had CD4 above 500 cells. There was a significant relationship between haemoglobin concentration and CD4 count in the infected children (p = 0.016). (Table IV). The correlation coefficient (R = 0.240) indicated a weak positive relationship existing between the two variables. The coefficient of determination (R2 = 0.058) indicated that little changes in haemoglobin concentration can be explained by CD4 count while regression coefficient (B = 0.001) indicated a positive effect of CD4 count on haemoglobin concentration. There is no significant relationship between CD4 count and absolute neutrophil count, p = 0.239 and between CD4 count and platelet count, p = 0.367.

Table 4: Relationship between levels of haematological parameters and CD4 count of the infected children.

Statistics	Haemoglobin	Neutrophil	Platelet
R	0.240	0.119	0.091
R^2	0.058	0.014	0.008
B	0.001	0.565	22.694
p value	0.016	0.239	0.367

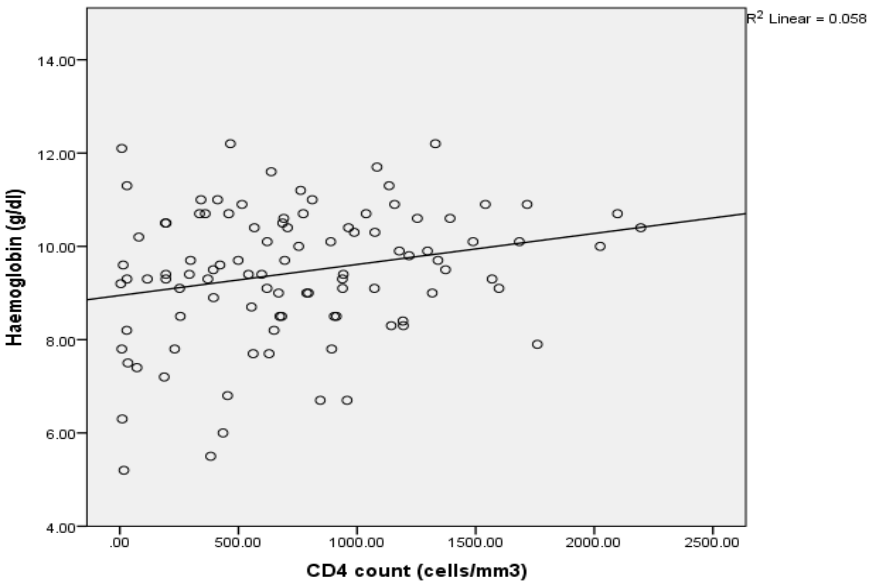
Table 5: Comparison of haematological parameters in male and female test subjects.

	Gender			
	Male	Female	Mann -Whitney	p-value
Parameters	Median (Mean rank)	Median (Mean rank)	U	
Neutrophil	2394.00 (48.05)	2472.50 (52.76)	1130.50	0.418
Platelet	325000.00 (52.53)	314500.00 (48.63)	1150.50	0.501
Haemoglobin	9.32 ± 1.67 *	9.55 ± 1.18 *	t = 0.789	0.432

There was a significant positive but weak relationship existing between haemoglobin concentration and CD4 count in the HIV positive ART-naïve children. (Fig 1)

The cluster of points does not follow any regular pattern indicating no significant relationship between neutrophil levels and CD4 count of the subjects. There was also no significant relationship between platelet levels and CD4 count of the HIV infected study participants

Figure 1: A scatter diagram showing the relationship between haemoglobin levels and CD4 count of the infected children.



DISCUSSION

A 60% prevalence of anaemia in this study is comparable to 64% prevalence in another study.[17]In both studies anaemia was diagnosed as hemoglobin less than 10g/dl. However, this 60% prevalence in this study was significantly higher than the ones reported by some authors who reported 28.1% and 35% respectively.[18-19]Good adherence to follow-up and general improvement in living conditions was attributed for the relatively lower prevalence. Pasha documented high prevalence rate of 89% although anaemia was diagnosed as haemoglobin concentration less than 12g/dl in their study.[20]

The difference in haemoglobin concentration of HIV-infected and uninfected children have also been documented previously.[17,21] Possible reasons are bleeding from possible gastrointestinal malignancy, severe infection, poor nutritional intake (iron, cobalamin, folate, iron, general), haemolytic anaemia, changes in erythropoietin synthesis and/or bone marrow suppression.
Also, the difference in the hemoglobin concentration between the genders was not significant statistically, although some authors reported a higher prevalence of anaemia in females.[22-23] Reasons for this finding are not clear however previous studies which reported higher prevalence of anaemia in females were done in adult females where menstruation, pregnancy and child birth may have contributed to iron depletion.

The positive but weak relationship between increasing age and haemoglobin concentration was also corroborated by Ferede.[21]No such relationship was found in the test controls. As the CD4 count increased, the haemoglobin concentration also increased. This finding is supported by other reports.[19,24]

The prevalence of neutropenia in this study was 18% in the test subjects and 14% in the test controls. This 18% prevalence was higher compared to the studies done by Amballie *et al* [25]which showed prevalence of 6% and 10% respectively. The difference may be due to variation in study population which were adult patients, study design method which was a 5 year retrospective review of case notes of patients. The increase or decrease in ages of both the infected and the test controls had no effect on the absolute neutrophil counts. This is not unexpected as ANC is not known to vary with age after infancy.

In this study, there was no significant relationship between neutropenia and CD4 counts. This was in contrast to the studies byCalenda[26]who reported significant association between neutropenia and CD4 counts. The 7% thrombocytopenia in the positive children was higher than a previous report in the study area that showed a prevalence of 5.9%. [19]An Indian study has also reported a lower prevalence (4.65%) of thrombocytopenia.[27] Higher results in this study may be due to the difference in the study population(which were children unlike the previous reports) or environmental factors as there was no statistical difference between the infected and the controls in our study. However, a prevalence of 20% in thrombocytopenia was reported from Iran.[28] The reason for this high prevalence of 20% may be attributed to the late presentation of their patients and the higher cut off value used to define thrombocytopenia(platelet count less than 100,000 per micolitre of blood while 50,000 was used in this study). Advanced HIV infection have been documented to worsen thrombocytopenia[29]

Reasons for the higher prevalence of thrombocytopenia in female infected children is not clear.There were no relationship between age and thrombocytopenia in this study. This was corroborated in the study by Alaei.[28]The CD4 count of the study participants did not show significant association with thrombocytopenia unlike a report from North West Ethiopia where reducing CD4counts was associated with increasing risks of thrombocytopenia.[19]

CONCLUSION

This study shows that anaemia is quite common among HIV positive ART-naïve children and was more prominent with decreasing age. It was also noted anaemia was a weak predictor of CD4 count.

DECLARATION

The authors declare that they have no competing interests.

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